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Efficacy of Different β-Lactams against an Extended-Spectrum β-Lactamase-Producing *Klebsiella pneumoniae* Strain in the Rat Intra-Abdominal Abscess Model

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Although standard-inoculum MICs were within the susceptible range for all compounds, cefoperazone, cefotaxime, and cefpirome were significantly less effective than imipenem or the combination of cefoperazone and sulbactam in the treatment of rat intra-abdominal abscesses due to an extended-spectrum β-lactamase-producing strain of *Klebsiella pneumoniae*.

The introduction and widespread clinical use of extended-spectrum cephalosporins have been associated with the discovery of plasmid-mediated enzymes capable of hydrolyzing these newer agents (2). The activity of these extended-spectrum β-lactamases against β-lactams which efficiently penetrate the cell membrane, such as cefotaxime, is often difficult to detect by standard-inoculum MIC testing (6) but is demonstrable when higher inocula are employed (2). This inoculum-dependent effect raises serious questions about the potential efficacy of cefotaxime and other extended-spectrum cephalosporins against infections caused by these organisms.

We have recently reported an outbreak of infection and colonization with organisms elaborating two distinct broad-spectrum β-lactamases at a chronic care facility (5). The more common of these two enzymes, YOU-1, a novel extended-spectrum enzyme with an isoelectric point of 5.57, conferred high-level resistance to ceftazidime (MIC = 256 μg/ml) but did not confer resistance to cefotaxime (MIC = 1 μg/ml) at a standard inoculum of 10^5 CFU/ml. We designed this study to compare the in vivo efficacies of various β-lactam antibiotics in the treatment of rat intra-abdominal abscesses due to a clinical isolate of YOU-1-producing *Klebsiella pneumoniae*.

K. pneumoniae 5657 was a clinical isolate from sputum. The presence of a TEM-related, plasmid-mediated extended-spectrum β-lactamase was confirmed as previously described (5). *Bacteroides fragilis* 2197 was a clinical isolate obtained from the stock collection of our laboratory. The antimicrobial agents used in these studies were kindly provided by the following manufacturers: cefotaxime and cefpirome, Hoechst-Roussel Pharmaceuticals, Somerville, N.J.; imipenem-cilastatin, Merck, Sharp and Dohme, Inc., West Point, Pa.; cefoperazone and sulbactam, Pfizer Inc., New York, N.Y. Ceftazidime (Glaxo Pharmaceuticals, Research Triangle Park, N.C.) was purchased from the hospital pharmacy. MICs were determined by a broth macrodilution method at standard inoculum (ca. 10^5 CFU/ml) and high inoculum (ca. 10^7 CFU/ml) in unsupplemented Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) (4). Abscesses were created by intraperitoneal placement of a gelatin capsule containing sterile rat cecal contents derived from grain-fed rats, killed *B. fragilis*, and a 1:100 dilution of an overnight culture of *K. pneumoniae* 5657 (ca. 10^5 CFU) in a 2:1:1 ratio. Rats weighing between 175 and 200 g were anesthetized with an intraperitoneal injection of ketamine sulfate (ca. 125 mg/kg) (Parke Davis, Morris Plains, N.J.). A small incision was then made in the left lower quadrant of the abdomen, and the infective capsule was placed directly into the peritoneal cavity. Treatment was begun by continuous infusion via the left internal jugular vein (7) 3 to 4 h after the placement of the capsule and continued for 3 days. Animals were randomly assigned to one of seven treatment groups: (i) untreated control, (ii) cefoperazone (600 mg/kg/day), (iii) cefoperazone (600 mg/kg/day) plus sulbactam (300 mg/kg/day), (iv) cefotaxime (400 mg/kg/day), (v) cefpirome (400 mg/kg/day), (vi) ceftazidime (400 mg/kg/day), and (vii) imipenem-cilastatin (300 mg/kg/day). On day 2 or 3 of therapy, blood samples were collected for the determination of serum antibiotic levels. Concentrations in serum were measured by high-performance liquid chromatography or by an agar well diffusion technique (1). After 3 days of therapy, animals were sacrificed at 2 h after discontinuation of the infusion (antimicrobial agent levels were undetectable in the serum at this point) by an intravenous injection of ketamine sulfate (25 mg/kg). The abdominal wall portion of the abscess was removed in a sterile fashion, weighed, homogenized in 2 ml of sterile saline, serially diluted, and inoculated in parallel onto brucella agar plates containing 5% horse blood (BBL Microbiology Systems) and MacConkey agar (BBL Microbiology Systems) plates containing ceftazidime (10 μg/ml). Bacterial titers in the abscesses were expressed as CFU per gram of abscess. Differences in bacterial titers among treatment groups were assessed by analysis of variance followed by Bonferroni's *t* test for multiple comparisons (3).

MICs of all of the antimicrobial agents tested are shown in Table 1. Substantial increases of MICs were seen for all compounds when the inoculum was increased to 10^7 CFU/ml. For imipenem and the combination of cefoperazone and sulbactam, the increase in MICs occurred in a graded fashion, with a marked decrease in turbidity occurring at 4 μg/ml for both and full clearing occurring at 256 μg/ml (cefoperazone plus sulbactam) or 16 μg/ml (imipenem). The

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TABLE 1. MICs of various agents for *K. pneumoniae* 5657

Antimicrobial agent	MIC (μg/ml) for *K. pneumoniae* 5657 at an inoculum of:	
	10^5 CFU/ml	10^7 CFU/ml
Cefoperazone	2	256
Sulbactam	32	
Cefoperazone-sulbactam (2:1)[a]	0.5	256
Cefotaxime	1	256
Cefpirome	1	>256
Ceftazidime	>256	
Imipenem	0.5	16

[a] MICs are micrograms of cefoperazone per milliliter.

other three β-lactams for which the increased inoculum was tested allowed full growth up until the respective MICs.

Serum antimicrobial agent concentrations and abscess bacterial titers for the treatment and control groups are listed in Table 2. With the exception of the ceftazidime group, levels in serum in all cases were 5 to 30 times the standard-inoculum MIC. Serum sulbactam concentrations were unmeasurable by high-performance liquid chromatography, presumably because of prolonged storage prior to the performance of the assay. A previous study in our laboratory revealed serum sulbactam concentrations of 5.9 ± 2.4 μg/ml for a sulbactam dose of 225 mg/kg/day and 9.5 ± 1.1 μg/ml for a dose of 500 mg/kg/day (8). Thus, the serum sulbactam concentration in our cefoperazone-sulbactam group was almost certainly well below the test organism MIC of this antibiotic. Imipenem- and cefoperazone-sulbactam-treated animals had reductions in the CFU per gram of abscess to levels significantly different from those of controls and all of the other treatment groups ($P < 0.05$ for all comparisons).

Our results suggest that expanded-spectrum cephalosporins may be less effective in the treatment of serious infections due to extended-spectrum β-lactamase-producing gram-negative bacilli than standard susceptibility tests would imply. In vitro studies indicated that the activity of these agents against *K. pneumoniae* 5657 was highly inoculum dependent. This organism exhibited a much less pronounced inoculum effect against imipenem, an agent against which extended-spectrum β-lactamases exhibit negligible activity. The inoculum effect did persist against cefoperazone in the presence of sulbactam, a β-lactamase inhibitor, but was qualitatively less dramatic. Increasing the sulbactam concentration was shown to further decrease the inoculum effect in a prior study (2). The dramatic improvement of the in vivo efficacy of cefoperazone in the presence of sulbactam suggests that the failure of treatment with cefoperazone alone was due at least in part to the presence of β-lactamase and may be due to a phenomenon similar to the inoculum effect observed in vitro. Therefore, it may be prudent to avoid extended-spectrum cephalosporins as single agents when treating serious infections due to these organisms.

TABLE 2. Intra-abdominal abscess treatment outcomes

Antibiotic	No. of rats	Mean serum antibiotic level (μg/ml) ± SD	$\log_{10}$ CFU/g of abscess ± SD
None	30		8.02 ± 1.02
Cefoperazone	11	13.5 ± 4.72	7.41 ± 0.74[a]
Cefoperazone-sulbactam	11	8.9 ± 3.22[b]	5.84 ± 0.95[c]
Cefotaxime	18	17.7 ± 8.42	7.26 ± 1.02[a]
Cefpirome	11	28.3 ± 2.06	7.80 ± 1.18[a]
Ceftazidime	10	19.4 ± 3.09	8.85 ± 0.64[a]
Imipenem	19	7.1 ± 2.08	4.99 ± 0.97[c]

[a] $P > 0.05$ for comparison with value for untreated controls.
[b] Concentration of cefoperazone.
[c] $P < 0.05$ for comparison with values for untreated controls, cefoperazone, cefotaxime, cefpirome, and ceftazidime.

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